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Environmental Enrichment of Laboratory Rodents: The Answer Depends on the Question

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Efforts to refine the care and use of animals in research have been ongoing for many years and have led to general standardization of rodent models, particularly with regard to animal housing, genetics, and health status. Concurrently, numerous informal practices and recommendations have been promulgated with the laudable intent of promoting general animal wellbeing through so-called enrichment of the cage environment. However, the variety of housing conditions fostered by efforts at environmental enrichment (EE) complicates the goal of establishing standardized or even defined environments for laboratory rodents. Many studies over the years have sought to determine whether or how various enrichment strategies affect the behavior and physiology of laboratory rodents. The findings, conclusions, and interpretations of these studies are mixed, particularly with regard to their application across rodent species, strains, genders, and ages; whether or how they affect the animals and the science; and, in some cases, whether the effects are positive, negative, or neutral in terms of animal wellbeing. Crucial issues related to the application of EE in research settings include its poorly defined effect on the animals, the potential for increased variability in the data, poor definition across labs and in publications, and potential for animal or scientific harm. The complexities, uncertainties, interpretational conundrums, varying conclusions, and lack of consensus in the EE literature warrant careful assessment of the benefits and liabilities associated with implementing such interventions. Reliance on evidence, professional judgment, and performance standards are crucial in the development of EE strategies.

Abbreviations: CCK2R-KO, cholecystokinin-2-receptor-deficient; Cyp1A1, cytochrome P450 1A1 gene; EE, environmental enrichment.

Efforts to refine the care and use of animals in research have been ongoing for many years and have led to general standardization of rodent care, particularly with regard to animal housing, genetics, and health status. Such standardization has resulted in more stable and better defined research subjects and likely has contributed to a substantial decrease in the number of rodents needed for research and testing.[15,16] However, efforts to promote the wellbeing of laboratory animals have concurrently fostered numerous informal practices and recommendations for provision of environmental enrichment (EE). The intended meaning of the term EE varies widely. For example, EE has been defined as the use of housing conditions that offer enhanced sensory, motor, and cognitive stimulation of brain neuronal systems in comparison with standard caging[13] and, alternatively, as adding biologically relevant features to the cage environment to facilitate or allow the performance of natural motivated behaviors.[53] Although these definitions are not mutually exclusive, the perspectives and probably the goals are clearly different.

EE paradigms typically incorporate features such as social housing, larger enclosures, and environmental complexity (for example, the presence of objects that can be manipulated, structures for climbing or exercise, foraging opportunities, hiding or nesting areas).[6,13] Thus, EE can take many forms. Even solid-bottom bedded cages, which are currently the standard method of housing rodents, may be viewed as providing enrichment as compared with historically common wire-bottom cages because rodents can use the bedding for the species-appropriate behaviors of burrowing and foraging, and the provision of bedding may reduce aggression.[3,5] However, some forms of EE can be associated with increased aggression.[7,23,24]

The variety of housing conditions fostered by efforts at EE complicates the goal of establishing standardized or even defined environments for laboratory rodents. Accurate monitoring and reporting of the animal environment, including all facets of the provision of EE, is a crucial component of scientific publications.[26] Even reporting the consistency or age of provision of EE is a consideration. For example, one study found long-term motivational differences between previously enriched mice after removal of the enrichment as compared with mice maintained under standard conditions, indicating a sustained behavioral effect of loss of enrichment.[39] The effects of EE are likely to vary depending on the animal model, assessment measures used, and experimental question. However, standardization of EE programs is problematic at present at least in part because of a lack of consensus regarding the definition and implementation of enrichment and its effect on both the animals and the science (Table 1).[5]

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Table 1. Questions and issues relevant to the use of environmental enrichment in rodents

	Question	Issues
1	What is enrichment?	Definitions of EE vary widely. Reliance on professional judgment in the context of experimental goals is crucial to the development of sound approaches to providing EE. Variation across rodent species and strains, together with the wide range of experimental goals, complicates the development of highly inclusive, prescriptive, or preemptive statements about EE.
2	What is enrichment intended to accomplish?	The goals of EE should be carefully defined, with measurable outcomes that benefit the animal in a significant way in terms of stimulation of positive species-typical behaviors and/or prevention of abnormal or undesirable behaviors.
3	How is an enriched environment related to standardized housing and a natural habitat?	Some components of the standard rodent environment might be considered to provide EE (for example, bedding, grilled cage tops, group housing, behavioral training), particularly for rodents maintained for a relatively short period of time.
4	Who should determine whether a given enrichment intervention benefits and/or does not harm the animal or the science?	EE should not be imposed unilaterally or arbitrarily by any of the stakeholders. Prior consideration of potential impact on research outcomes is essential. Evidence should be available to document that specific forms of EE will not confound experimental outcomes and/or will alleviate harm that occurs in the absence of the EE. A corollary of this point is that funding is typically not available to support studies of the impact of EE on the animals or the research.
5	What is 'enough' enrichment, and how can that be measured?	Common indices of animal wellbeing are variables such as food intake, growth, and appropriate breeding performance, which are commonly satisfactory under standard rodent housing conditions. Determining the amount or form of EE needed to produce a positive impact without causing harm is a complex task.
6	What are the potential negative consequences of EE?	In some situations, EE interventions may cause overt harm to animals, influence statistical considerations, confound interpretation of the data, or impede the replication of findings across laboratories.
7	What are potential negative consequences of social housing?	Social animals can be highly territorial and/or develop hierarchical relationships that may adversely affect both high- and low-ranking animals. The presence of both dominant and submissive individuals also creates variability among animals in the group.

General Approaches to the Study of EE

Many studies over the years have sought to determine whether or how various EE strategies affect the behavior and physiology of laboratory rodents. The findings and conclusions of these studies are mixed, particularly with regard to the application of EE interventions across rodent species, strains, sexes, and ages; whether or how they affect the animals and the science; and, in some cases, whether the effects are positive, negative, or neutral in terms of animal wellbeing. A brief overview of the general types of approaches that have been used to study EE illustrates the complexity that pervades its use.

The most basic approach to studying EE is to evaluate the effect of specific EE interventions on physiologic or behavioral measures in normal animals. Several such studies report, for example, that EE either changes the mean values of body weights, organ weights, or hematologic parameters or does not influence mean values but increases variation in mean values of some measured variables.[14,19,47,69,70] As an example of a behavioral study, a comparison of C57BL/6 and 129S6/SvEv mice found that housing in an enriched compared with a standard environment increased exploratory activity in the plus-maze test and reduced habituation in the locomotor activity test in C57BL6 mice, whereas 129S6/SvEv mice showed increased hot-plate latencies and reduced aggression.[1] Furthermore, EE accentuated strain differences in the plus-maze, locomotor activity, hot plate, and forced swim tests, whereas strain differences in the plus-maze and resident-intruder tests were not retained across environments.[1] The effect of enrichment on responses in rats also varies with both the rat strain and the form of enrichment used.[31,35] However, many studies find no effect of EE on facets of physiology and behavior. For example, one study evaluated the effect of nesting material on mice and detected no major differences in behavioral and physiologic measures, concluding that supplying nesting material does not jeopardize the outcome of experiments.[74] However, another study in mice found that core temperature and locomotor activity were affected by bedding type and amount, whereas metabolic rate was not, leading to the conclusion that variation in bedding material may affect toxicologic and pharmacologic studies in which the measures are influenced by body temperature.[20]

A related experimental approach to the assessment of EE evaluates the effect of cage size and housing density on normal animals with and without additional enrichment. A recent study complements and extends this approach by reporting that the olfactory environment associated with 2 different cage types altered both neuroanatomic features of the mouse olfactory bulb and mouse aggressive behavior.[51] Studies like these often identify complex interactions between cage type, housing density and EE, with additional influences that include gender, cage size, and the specific measure of assessment used (for examples, see references 27 and 82). Therefore, the identification of an optimal cage density or EE strategy that can be uniformly applied across diverse situations and members of a species will be a difficult task.[38]

Another approach to the assessment of EE evaluates its effect on disease models, as compared with normal animals. As in studies of normal animals, findings from these studies are also complex, as illustrated by the following examples. In a study of hamsters that were housed individually, enrichment and larger cage size were associated with lower mean baseline rectal temperatures, but with a greater mean febrile response to injected lipopolysaccharide, with no effect on variability.[37] Therefore, in this study, cage size appeared to influence thermoregulatory homeostasis differently under basal conditions and in response to an experimental manipulation.[37] As another example of this complexity, cholecystokinin-2-receptor-deficient (CCK2R-KO) and control mice housed under EE or standard housing showed significant genotype-by-environment interactions in a number of behavioral tests.[2] As compared with genetically intact littermates, CCK2R-KO mice had higher measures of anxiety and restraint-induced analgesia and worse performance in the water maze under standard but not under enriched conditions.[2] Mice housed in enriched, but not in standard, conditions showed a genotype-dependent phenotype in the hot-plate, rotarod, and locomotor activity tests; for some tests, these effects were gender-dependent.[2]

In other models, EE may have similar effects on normal and abnormal animals. For example, in a model of Alzheimer disease, EE had comparable effects in both transgenic and wildtype mice, generating more exploratory and locomotor behavior without affecting measures of learning and memory.[21] Another study determined that EE did not interfere with the response of mice to infection with *Mycobacterium avium* for as long as 20 wk, as assessed by the bacterial load in the spleen and lung, the number and activation status of the main cell populations of the immune system, and the serum concentration of interferon γ.[52] As a final example, housing in standard or enriched cages did not affect either mean values or variability in behavioral measures after the administration of the anxiolytic drug diazepam.[4]

These selected examples, like all other studies of this type, evaluate only a limited number of conditions, parameters, and strains, making broad conclusions seem unwarranted in light of these clear limitations in scope. The thoroughness of such studies and the associated scope of their conclusions require careful scrutiny before specific forms of EE are implemented. For example, a consortium of experienced behavioral neurobiologists investigated whether subtle changes in cage environment could affect outcomes in behavioral tests; they identified several significant and distinct genotype-by-environment-by-test interactions and showed that strain phenotype distribution patterns for some measures could be reversed depending on the form of enrichment used, whereas other measures were not affected by the enrichment condition.[73]

Defining the Adequate Environment

Captive environments can limit an animal's opportunity to engage in some aspects of its normal behavioral repertoire; poor adjustment to such limitations may alter normal physiology and lead to the development of abnormal behaviors.[18] However, animal wellbeing, as reflected by normal growth, development, and reproduction with low likelihood of injury, illness, distress, or maladaptive behavior, can exist even in housing situations in which the animal cannot perform its entire repertoire of species-appropriate behaviors, particularly if the animal will be maintained for a relatively short portion of its lifespan.[48] Furthermore, the behavioral needs of animals that have been bred for generations under laboratory conditions may differ substantially from those of similar wild or ancestral species, and laboratory species, like other domestic species, have probably adapted to the confined and controlled conditions in which they live.[49,64] In addition, the history of individual animals can affect their relationship to the captive environment. For example, in a study of mice reared in enriched or standard cages, previously enriched mice showed more behavioral abnormalities when moved to standard conditions than did mice reared from birth in such conditions.[39] Previously enriched mice also showed higher motivation to access enrichments.[39]

Stereotypy is repetitive, unvarying, apparently purposeless behavior that can occur in people and animals.[18,22] Stereotypy often occurs together with general changes in patterns of responsiveness that could alter some experimental measures (for example, extinction learning, home cage activity, response latency, behavioral switching).[18] However, identifying enrichment that can prevent or alleviate these abnormalities is itself a complex task. For example, when deer mice were provided with either a functional or locked running wheel from the time of weaning and evaluated for stereotypy at 30 and 45 d of age, they showed no significant effect of exercise on stereotypy and no association between wheel running and stereotypy.[56] Therefore, the opportunity for exercise, which would appear to offer EE, does not prevent the development of stereotypy under these conditions.[56] In rats, the daily feeding schedule and access to a running wheel interact to influence the development of gastric ulcers.[54,87] Although correlations between stereotypy and perseveration have been reported for some species,[18] a study designed to examine stereotypy and perseveration in mice found that EE significantly reduced stereotypic behavior but did not significantly affect perseveration, and performance in a perseveration task did not correlate positively with stereotypy.[22]

Even seemingly simple efforts to provide opportunities for species-appropriate behaviors have complex aspects to their implementation. For example, even the location of a plastic nest box within the cage can influence whether mice use or avoid it.[36] Furthermore, the type of nesting material that is available to the animal influences its enrichment value. Providing naturalistic nesting materials, as compared with less natural substitutes, allows laboratory mice to construct complex dome-shaped, multilayered nests similar to those of wild mice.[25] Although provision of nesting material may reduce aggressive behavior in some strains of mice,[75-77] providing a shelter can increase aggression and physiologic indices of stress in other strains.[23,43,75]

Complex and unexpected effects of EE on research variables are also possible. For example, mice given cotton balls as a form of EE showed liver damage and induction of the cytochrome P450 1A1 gene (*Cyp1A1*), which typically is triggered by exposure to dioxins and dioxin-like compounds; mice with no exposure to cotton balls had no liver damage, low levels of *Cyp1A1* transcript, and undetectable levels of CYP1A1 protein.[68] These data suggest that cotton balls are potentially contaminated with dioxins or dioxin-like compounds (or both) through the production and bleaching process and underscore how providing untested enrichment modalities to animals in research facilities can have unintended effects.[68]

Similar conundrums pervade the assessment of reproductive performance as a measure of housing suitability and the effect of EE. For example, one group reports that EE was associated with fewer pups born, fewer litters per dam, and an earlier age-related decline in production in breeding females, did not significantly affect breeding index (number of young weaned per dam per week), and showed a complicating interaction with type of caging system used.[69,71] In contrast, another group reports that EE improved reproductive performance in that pups from nonenriched cages weighed less than pups from enriched cages, and fewer survived to weaning age.[82,83] In another study, rats that were housed in a complex environment during gestation and parturition and after delivery were leaner, maintained a constant postpartum weight, and had heavier but fewer offspring as compared with rats housed under standard conditions.[65] An important question with regard to studies of this type is whether the basal condition is inadequate or harmful to breeding success and whether the statistically significant changes associated with the enriched conditions are biologically or clinically significant.

Exposure of animals of many species to humans and novel situations and objects early in life can make them easier to handle in a research setting and reduce the negative consequences of novel situations and objects they may experience later in life. For example, providing toys and food treats in the environment may make rabbits more sociable toward their human caretakers without adversely affecting reproduction.[11,28,29,32,58] Although potential effects of these interventions on research outcomes have not been evaluated, the effect is likely to be negligible in many cases but may be significant in others. Furthermore, although positive human contact and exposure to novel objects and situations may be feasible for some species in some situations, their application to large numbers of animals (particularly rodents) can be problematic from the perspective of disease risk and personnel time. Adoption of any form of EE requires the development of satisfactory approaches to managing practical obstacles to its implementation.[55]

Enriched and Standardized Environments: Considerations of Experimental Design

Several recent publications have questioned the design and reporting of animal research and the validity of animal models, particularly with regard to the ability of such studies and models to predict clinical efficacy in drugs selected for human trials.[33,42,57,59] With regard to validity, some work has shown that environmental standardization may generate spurious findings that either cannot be confirmed or can be confirmed only in animals maintained in specific highly controlled environments.[61,62,86] An analogous issue arises in human clinical trials conducted in academic settings; the same benefits that are identified in randomized controlled trials are not always achieved in the real world of day-to-day clinical practice.[67,81,85] However, in both animal research and human clinical studies, the importance of broad applicability of findings across diverse populations depends on the objectives of the studies in question. Validation in diverse populations may not be necessary or even appropriate for many experimental questions. Support for this perspective may be found in the emergence of personalized medicine, where even minor subject differences can influence disease susceptibility or the efficacy and/or toxicity of therapies. Nonetheless, sound experimental design, valid approaches to data analysis, and comprehensive accurate reporting of methods and results are essential to the advancement of both generalized and personalized medicine.

With regard to statistical design, conditions that contribute to greater interindividual variability in measured parameters can potentially require the use of greater numbers of animals to achieve reasonable statistical power.[70] Changes in the animal or its environment can influence many measures of animal behavior and physiology, potentially altering the basal conditions, increasing variability across animals or labs, or even influencing experimental outcomes. For example, even subclinical microbial infections can alter the behavioral or immune status quo in animals, thereby obscuring, amplifying or even changing the effects of experimental challenges. However, defining such effects is difficult. For example, a study of male and female mice of 4 inbred strains indicated that EE can affect experimental results, does not necessarily improve wellbeing, and may create conflicts between achieving ‘refinement’ compared with ‘reduction.’[72] In contrast, another report found that EE does not increase individual variability in behavioral tests or the likelihood of obtaining conflicting data in replicate studies and concludes that housing conditions of laboratory mice can be enriched (‘improved’) without affecting the results.[84] In any case, carefully controlled and well-defined conditions can be crucial to the detection and measurement of subtle and complex biologic signals, although in some situations and for some experimental questions, defined diversity may be advantageous.[61,62,86]

Controlling interlaboratory variation and obtaining consistent results can be difficult even when standardization is part of a study by design.[10,30,78,80] For example, in a study to assess the consistency of the behavioral effect of different housing conditions across laboratories and experimenters, absolute values measured for some tests varied significantly; in contrast, the relative effects of enriched compared with standard housing were consistent.[41] This consistency led the authors to conclude that behavioral phenotyping is reliable if appropriate standardization and controls are used. In addition, a comparison of recently collected and historic behavioral data in mice found that phenotypic drift over decades has been minimal for most of the behaviors examined.[79] For example, strain differences in ethanol preference and locomotor activity have been highly stable, with most strain correlations as high as or higher than for brain weight.[79] However, strain differences in anxiety-related behavior vary markedly across laboratories, including within the same laboratory after relocation to another site within a university.[79] In a related study, strain effects were generally large, and key measures for some tests were essentially the same across laboratories.[80] Using a higher benchmark for significant effects may reduce the likelihood of having inconsistent findings across laboratories.[30] A complementary approach is to determine which behavioral tests are generally reliable or variable across labs and to develop new tests that yield stable results across sites.[30,78]

The Value of EE in Scientific Discovery

For some experimental questions, the research application of EE undoubtedly has contributed to important insights into disease mechanisms and recovery from the damage caused by disease. Exposure to EE has been associated with altered brain neurogenesis, chemistry, and function; benefits have been reported for the treatment of depression and mental retardation, vulnerability to drugs of abuse, and cognitive and other functional deficits in models of aging, stroke, neurodegenerative diseases, and epilepsy.[9,13,40,45,46,50,63,66] In addition, failure to recognize that rats and mice used in biomedical research are often sedentary, obese, and even glucose-intolerant in the ‘control’ state can confound data interpretation and study conclusions.[9,44]

Despite its experimental value in some situations, careful interpretation is essential when formulating conclusions about the effect of EE on experimental outcomes. For example, in experimental settings, both the administration of antidepressant drugs and provision of a stimulating environment positively influence cognition and neuronal plasticity.[8] However, long-term treatment with various antidepressant drugs increases putative markers of these processes in key brain regions whereas EE does not, indicating that their influence is not identical, despite similar effects on cognitive performance.[8] Furthermore, despite considerable evidence that EE can modulate brain development and promote

Table 2. Key concepts relevant to changing housing conditions for research animals

1	Providing adequate animal care may not require EE, nor does provision of EE necessarily improve animal wellbeing.
2	Animals' environmental preferences are not a guideline to their wellbeing and can be physically detrimental.
3	In many cases, neither laboratory animal science experts nor researchers can be certain whether altering a standard rodent environment compromises animal wellbeing or research results. When either outcome is in question, EE should not be mandated by the institution or oversight agencies.
4	Alterations in housing that clearly promote better health, reproduction, and fitness benefit both the animals and those who use and care for them. However, attempting to improve emotional states that cannot be reliably identified or measured may not benefit or may harm the animals or the research.
5	Variability can be difficult to control both within and between laboratories. The potential for small environmental differences to significantly affect research results should not be underestimated.

Adapted from reference 6.

recovery from brain damage, consensus has not been reached concerning which aspects of enrichment are either crucial or optimal with regard to causing those effects.[6,14,50] For example, in a transgenic mouse model of Alzheimer disease, the beneficial effects of access to a running wheel appeared to depend on when the wheel was provided during development of the disease.[60] Furthermore, wheel-running was inversely correlated with stereotypy and positively correlated with plaque burden, leading to the conclusion that wheel-running may have stereotypic qualities and may be symptomatic of brain pathology, rather than protective.[60] In some situations, social isolation, as compared with or in addition to an enriched environment, may provide a superior model.[34,60]

Conclusions

Achieving optimal housing conditions for animals is a laudable goal, whether applied to research settings, agricultural production, zoos, work animals, and even pets. As such, the consideration of interventions to improve animal wellbeing is warranted under many if not most circumstances. However, the complexities and uncertainties that surround the application of changes in housing in the research environment warrant careful consideration of the benefits and liabilities associated with such interventions and suggest that reliance on professional judgment and performance standards is advantageous as compared with rigid requirements for EE. Our review of these issues, together with our perspective as scientists who use animals in research and want to use them humanely, leads us and others to a number of conclusions and recommendations (Table 2). Crucial issues related to the application of EE are its undefined effect on the animals, the potential for increased variability in the data, poor definition across laboratories and in publications (potentially contributing to discrepant results across laboratories), potential for harm to the animal or the study, and the relative costs and benefits associated with adequate, optimal, and preferred housing. In addition, the response of even animals of the same species to their environment is influenced by many factors, including genotype, sex, and age.[49,53,74]

Studies of the effect of the environment on animals are always limited by design, leaving open the question of whether other crucial but unmeasured parameters are (or are not) changed or made more variable by changes in the environment. These limitations support a careful evaluation of EE interventions prior to implementation in a research setting to determine that they actually improve animal wellbeing, do not create an environment that reduces animal wellbeing or endangers animals, or either do not alter or perhaps improve experimental results.[5] As stated by others, "the provision of enrichment should be evaluated in the context of the health of the animal and research goals on a case-by-case basis" and "ultimately, the decision to include a particular type of enrichment should be based on a consideration of the safety of the animal and the staff, whether the enrichment has a demonstrable beneficial effect on the animal, and whether the potential effects of the enrichment are experimentally relevant."[5] Furthermore, "minor cage supplementation intended for improvement of animal well-being may alter important aspects of an animal's physiology and development in a manner not easily predicted from available research" and "we do not understand the mechanisms by which rodents respond physically to environmental changes sufficiently to implement them in a knowledgeable manner."[6] Because the same enrichment design can have positive, negative, different, or no effect, depending on the strains used and the variables studied, considerable forethought is necessary before the introduction of enrichment designs into experimental plans.[70,72] Such complexity and potential for adverse outcomes could directly jeopardize animal wellbeing, increase the number of animals needed, alter experimental results, and influence conclusions, particularly when comparing different strains of rodents.

Animal preferences should not be viewed as the ideal determinant of what best promotes their wellbeing because animals, like people, can make poor choices.[6,12,17] For example, some strains of mice will choose to drink an ethanol solution rather than plain water, rodents will choose to self-administer psychoactive drugs, and given a choice between a balanced diet and nutritionally deficient but highly palatable treats, many (most?) animals will choose the treats. A review that assessed 40 studies published between 1987 and 2000 concluded that mice 'prefer' a more complex cage compared with a standard cage because they will work for access to cages with shelter and raised platforms.[53] However, a key issue is not whether EE is preferred by animals, but whether it is essential or should be mandatory, assuming standard housing is adequate to promote animal health.

Broad institutional or regulatory mandates for EE should be avoided. Defining what is beneficial and desirable for an animal is a complex task, and the determination that a particular environmental modification is not overtly harmful to an animal does not necessarily indicate that it is desirable. An intervention that has a beneficial effect on animals of one species or strain may cause harm in others. Furthermore, even the previous experiences of an animal can significantly affect whether it responds positively or negatively to a particular item or situation. The design and implementation of EE should be based on solid data indicating that the enrichment intervention will enhance animal wellbeing without jeopardizing experimental design or outcomes. EE should be designed, assessed, and implemented based on the combined judgment of all professionals involved. IACUCs, husbandry personnel, and research staff should work together to provide an adequate environment that meets animal and research needs yet is practical, well defined and controlled; this decision should be informed by scientific data. In addition, scientists should provide accurate and comprehensive descriptions of the cage environment in publications of research data.[26] Together, these stakeholders should be able to determine which enrichment strategies could and should be used in conjunction with standard animal housing based on scientific data and experimental goals.

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